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Epidemiological characterisation of *Neisseria gonorrhoeae* isolates from the Far East

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SUMMARY One hundred strains of *Neisseria gonorrhoeae* (including 30 penicillinase producing (PPNG) strains) originating from Korea were characterised by plasmid analysis, auxotyping, and serogrouping. Eighty per cent of the isolates possessed the conjugative 24·5 megadalton (Mdal) plasmid. A novel 7·8 Mdal plasmid was present in four isolates (one PPNG and three non-PPNG strains). Seventy five per cent of all the strains tested were wild type and belonged to serogroup WII, while 20% were proline requiring and belonged to serogroup WII. Two of the remaining strains were tyrosine auxotrophs, while another strain was arginine requiring; these three strains carried the conjugative plasmid and belonged to serogroup WII.

Introduction

Since the emergence of penicillinase producing *Neisseria gonorrhoeae* (PPNG) in 1976,[1-3] the β-lactamase R-plasmids have been useful as additional epidemiological markers for tracing the possible geographical source of PPNG. The R-plasmid with a molecular weight of 3·2 megadaltons (Mdal) was associated with PPNG isolates from West Africa, while the 4·4 Mdal R-plasmid was associated with the Far East.[4-6] The 24·5 Mdal plasmid had the ability to mobilise R-plasmids conjugally,[7] and it was found more commonly in about 34% of the isolates from the Philippines.[8] Recent reports, however, showed that new plasmid patterns of *N gonorrhoeae* have appeared in various geographical localities.[9-14] Auxotyping has provided useful information on the epidemiological characteristics of *N gonorrhoeae* strains from various sources.[15-17] The 24·5 Mdal plasmid was found previously only in wild type or proline requiring gonococcal isolates, but recent findings showed that the conjugative plasmid could be found in other auxotypes like arginine (ornithine) requiring isolates[10 11] and in a proline plus methionine ($Pro^- + Met^-$) requiring strain.[18]

In this study a new serogrouping technique[19-23] was used in conjunction with auxotyping and plasmid analysis to characterise the gonococcal isolates from Korea.

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Materials and methods

One hundred isolates of *N gonorrhoeae,* including 30 strains of PPNG isolated from US military personnel in the Republic of Korea, were kindly provided for the study by the Walter Reed Army Institute of Research, Washington DC, USA.

The β-lactamase production of each gonococcal isolate was retested by using chromogenic cephalosporin (Glaxo Laboratories)[24] and a starch paper technique.[25] After reconfirming the identity of the gonococcal isolates by colonial morphology, oxidase test, and sugar utilisation patterns we stored all the cultures at −20°C in defibrinated rabbit blood pending further laboratory investigation. Included in the study were a reference PPNG strain carrying 2·6 Mdal and 3·2 Mdal plasmids (CDC 82-057010); an *Escherichia coli* strain carrying a gonococcal 3·2 Mdal plasmid; gonococcal strains (CDC 65-082) carrying a 24·5 Mdal plasmid, 79C′ carrying a 7·8 Mdal plasmid, and an African strain (LUNG 10) carrying 2·6 Mdal and 4·4 Mdal plasmids.

Plasmid deoxyribonucleic acid (DNA) from the cleared lysate was precipitated with ethanol and then subjected to agarose gel electrophoresis.[26] The gel was stained in a solution of ethidium bromide in water (1 μg/ml) for 30 minutes. The plasmid DNA bands were visualised with a short wave ultraviolet

light source (FOTO-UV* System, Fotodyne, New Berlin, Wisconsin, USA), and photographed with a Polaroid MP.4 land camera (Polaroid Corporation, Cambridge, Massachusetts, USA). Plasmids of known molecular weight were included as standards. Nutritional growth requirements of each gonococcal isolate were determined on gonococcal genetic medium.[16 17]

Serological grouping based on W antigens of gonococci was carried out using coagglutination methods.[20-23]

Results

Table I shows that a 4·4 Mdal plasmid was present in all the 30 PPNG strains tested. Of the 100 strains from Korea a conjugative 24·5 Mdal plasmid was present in 80, 29 of which were PPNG strains. The relation between plasmid content and auxotype showed that the 24·5 Mdal plasmid was present in three strains with auxotypes other than wild type and proline requiring; two of these strains were rare tyrosine requiring isolates, while the remaining strain was arginine requiring. The 7·8 Mdal plasmid, which is also rare, was present in four isolates in the study, one of which was a PPNG strain.

*Use of trade names is for identification only and does not imply endorsement by the Public Health Service or by the US Department of Health and Human Services.

TABLE I *Relation between plasmid content and auxotype of Neisseria gonorrhoeae isolates from Korea*

Plasmid profile (molecular mass in Mdal)	*No of isolates with following auxotype:*				
	Wild	*Pro⁻*	*Tyr⁻*	*Arg⁻*	*Total*
2·6	9	10			19
2·6 + 24·5	38	7	2	1	48
2·6 + 7·8 + 24·5		3			3
2·6 + 4·4	1*				1*
2·6 + 4·4 + 24·5	28*				28*
2·6 + 4·4 + 7·8 + 24·5	1*				1*
Total	77	20	2	1	100

*PPNG strains.
Mdal = megadaltons.

TABLE II *Relation between auxotype and serogroup of PPNG and non-PPNG isolates from Korea*

No of isolates with:			
Auxotype	*Serogroup WI*	*Serogroup WII*	*Total*
Wild Type	2	45 (30)*	77
Pro⁻		20	20
Tyr⁻		2	2
Arg⁻		1	1
Total	2	98	100

*There were 30 PPNG strains, all of which were wild type and belonged to serogroup WII.

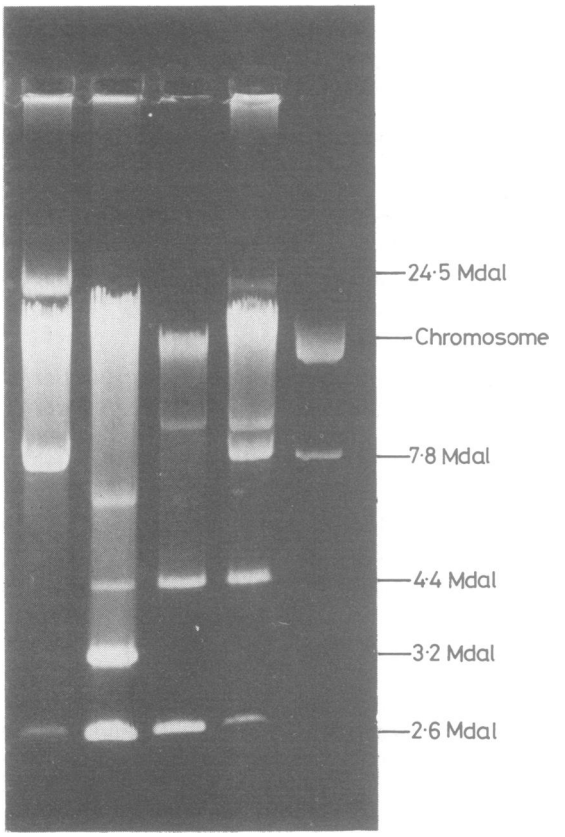

FIG 1 *Agarose gel electrophoresis of crude lysates of isolates of Neisseria gonorrhoeae: column 1—Korean non-PPNG strain, CDC 82-057912; column 2—reference strain 82-057010; column 3—reference strain LUNG 10; column 4—a Korean PPNG strain, CDC 82-077028; and column 5—reference strain, CDC 79C'. (Mdal = megadaltons.)*

Figs 1 and 2 show 0·7% agarose gels of electrophoresed crude lysates of representative strains.

The results of the serogroups of the *N gonorrhoeae* strains in relation to their auxotypes are summarised in table II. Of the 100 strains, 98 belonged to serogroup WII. The only two strains that belonged to serogroup WI were non-PPNG, and their auxotype was the wild type.

Discussion

The findings in this study indicate new plasmid and auxotype patterns in strains from the Far East. It is noteworthy that about 80% of gonococcal isolates from Korea possessed the conjugative 24·5 Mdal plasmid, whereas fewer isolates from the Philippines possessed this type of plasmid. Recent reports have

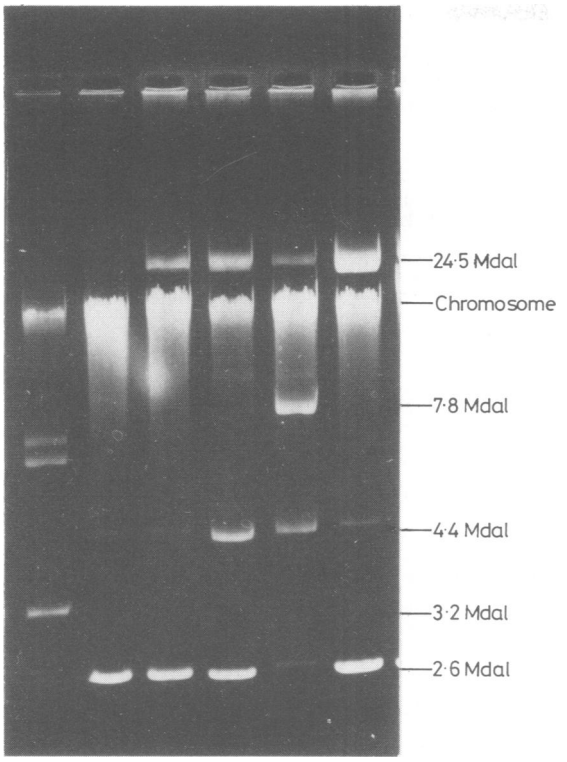

FIG 2 *Agarose gel electrophoresis of crude lysates of gonococcal isolates: column 1—reference 3·2 Mdal plasmid in Escherichia coli; column 2—a Korean non-PPNG strain (CDC 82-057931); column 3—a Korean strain CDC 82-057934; column 4—reference strain, CDC 65-082; column 5—a Korean strain CDC 82-077028; and column 6—a Korean non-PPNG strain, CDC 82-057882. (Mdal = megadalton.)*

suggested genetic exchange within the gonococcal population.[10-14 18 27] The 24·5 Mdal conjugative plasmid, which was previously present only in the wild type or proline requiring isolates, was reported to be present in one ornithine requiring strain from Canada,[10] three arginine (ornithine) requiring strains from Holland,[11] and one proline plus methionine requiring strain from Britain.[18] In this study we found an additional three strains, two of which had an unusual tyrosine requiring auxotype with the one remaining strain having an arginine requiring auxotype.

It is interesting that four of the 100 strains from Korea possessed a newly described 7·8 Mdal plasmid. The fact that this 7·8 Mdal plasmid was present in both PPNG and non-PPNG strains suggests that the plasmid does not code for β-lactamase. In a previous study from our laboratory only three strains of *N gonorrhoeae* were found to possess the novel 7·8 Mdal plasmid (Johnson *et al*, unpublished data). van Embden *et al* isolated a PPNG strain in Holland carrying a 7·5 Mdal cryptic plasmid which might be identical to our 7·8 Mdal plasmid.[27]

The predominant auxotypes in this study were wild type (77) and proline requiring (20) (table II). All the 30 PPNG strains were the wild type and belonged to serogroup WII, which had a total of 98 strains. These findings are in accord with other reports which indicated that serogroup WII is predominant in the Far East, while serogroup WI is predominant in West Africa.[14 19 22]

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